BIOLOGICAL CONTROL OF *DIPLODIA CORTICOLA* BY A NON PATHOGENIC *FUSARIUM TRICINCTUM* STRAIN. G. Campanile[1], A. Ruscelli[1], A. Moretti[2], S. Somma[2], A. Ritieni[3] and N. Luisi[1]. *[1]Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. [3]Dipartimento di Scienze degli Alimenti, Università di Napoli "Federico II", Via Università 100, 80055 Portici, NA, Italy. E-mail: antonio.moretti@ispa.cnr.it*

A *Fusarium tricinctum* (Corda) Sacc. strain isolated from the buds of *Quercus pubescens* Willd. in declined oak woods reduces incidence of *Diplodia corticola* A.J.L. Phillips, Alves & Luque, a causal agent of cankers, vascular necrosis and dieback in various oak species. Although non pathogenic *Fusarium* strains have been often used to control Fusarium wilts, scant information is available on the biological control of *D. corticola* by members of this genus. The main objective of this research was to identify possible secondary metabolites produced by *F. tricinctum* involved in the biocontrol of *D. corticola*. Fungal extracts of the *in vitro* rice kernel culture of *F. tricinctum* were analyzed by using High Performance Liquid Chromatography (HPLC), showing that this strain was able to produce high levels of enniatin B, enniatin B1, enniatin A1 and enniatin A (5050 µg/g; 4750 µg/g; 1860 µg/g, and 240 µg/g, respectively). *In vitro* experiments showed that enniatins revealed a low antifungal activity on *D. corticola* since they did not significantly reduce the growth of this fungus compared the control. However, in *in planta* experiments, enniatins inoculated in seedlings of *Q. cerris* L. and *Q. pubescens* Willd showed to be biologically active against *D. corticola* while they were not phytotoxic toward the plant seedlings.

MOLECULAR TOOLS TO MONITOR A BIOCONTROL AGENT AND TO ASSESS ITS ENVIRONMENTAL IMPACTS. V. Edel-Hermann, C. Steinberg, N. Gautheron, S. Brenot, S. Aimé and C. Alabouvette. *UMR Microbiologie du Sol et de l'Environnement, INRA, Université de Bourgogne, 17 rue Sully, 21065 Dijon Cedex, France. E-mail: veronique.edel@dijon.inra.fr*

The use of non pathogenic strains of *Fusarium oxysporum* has been proposed to control Fusarium diseases responsible for severe damages in many crops of economical importance. In order to monitor the biocontrol strain Fo47 after application in greenhouse and field, we have developed a specific molecular tool based on a sequence characterized amplified region (SCAR). Primers targeting a 210-bp fragment were designed to set up a real time PCR assay allowing the identification of the biocontrol strain and its quantification in environmental samples. The assay enabled specific detection of the strain in soils without cross detection of autochthonous populations of *F. oxysporum*. After its introduction in soil, a biocontrol agent will interact with all the biotic components and might have side-effects on the soilborne microflora. In order to assess the non-target effects of the strain Fo47, we have monitored the microbial community structures for one year following the introduction of the biocontrol strain in two different soils. Bacterial and fungal community structures were assessed using a molecular fingerprinting method based on terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S and 18S ribosomal DNA. In both soils, the community structures of both microbial components were not significantly affected by the introduction of Fo47, indicating that the microbial balance was not displaced by the biocontrol strain.

CONTROL OF *FUSARIUM GRAMINEARUM* WITH ANTIFUNGAL PLANT PREPARATIONS AND THEIR EFFECT ON MYCOTOXIN CONTAMINATION OF WHEAT. H.R. Forrer, A. Hecker, E. Jenny, T. Bucheli, F. Wettstein and S. Vogelgsang. *Research Station Agroscope Reckenholz-Tänikon ART, Reckenholzstrasse 191, 8046 Zurich, Switzerland. E-mail: hans-rudolf.forrer@art.admin.ch*

Fusarium graminearum (FG) is the most prevalent Fusarium Head Blight (FHB) fungus in Switzerland. To reduce the risk of FHB infection and mycotoxin contamination in wheat grains, chemicals are a possible remedy. However, the efficacy of fungicides is limited and their use is prohibited in organic wheat production. As an alternative, we selected plant preparations demonstrating sufficient antifungal properties in our previous field trials with late blight on potatoes. *In vitro* and *in vivo* bioassays served to determine the efficacy of these antifungal plant preparations (APP) against FG. In 2006 and 2007, the most active APPs, *Rheum palmatum*, *Frangula alnus* and preparations of the Chinese gall (*Galla chinensis*), were used in field trials with artificial FG infections. In both years, FG incidence on ears was significantly reduced by the best APP. The reduction was as good as that following applications with Pronto Plus®, a fungicide mixture of tebuconazole and spiroxamine. In 2006 or 2007, the APP treatment reduced the deoxynivalenol content by 67% and 50%, whereas the chemical reduced it by 59% and 52%, respectively. In 2006, mean concentrations of 2 ppm nivalenol (NIV) and 2.4 ppm zearalenone (ZON) were measured in the untreated check. The APP and the fungicide treatment reduced the NIV concentration to 0.6 ppm and those of ZON to 0.9 ppm or 1.3 ppm. In 2007, very low concentrations of NIV and ZON were detected. In conclusion, the best APP treatment proved to be as effective as the synthetic fungicide.

DETERMINISM OF THE BIOCONTROL CAPACITY OF A STRAIN OF *FUSARIUM OXYSPORUM*: IDENTIFICATION OF GENES EXPRESSED DURING INTERACTIONS WITH TOMATO. F. L'Haridon[1], S. Aimé[1], B. Scherm[2], Q. Migheli[2], C. Alabouvette[1] and C. Olivain[1]. *[1]UMR Microbiologie du Sol et de l'Environnement, INRA Université de Bourgogne, BP 86510, 21065 Dijon Cedex, France. [2]Dipartimento di Protezione delle Piante, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: chantal.olivain@dijon.inra.fr*

The main objective of this study was to identify fungal genes involved in the protective capacity of some strains of *F. oxysporum*. The approach consisted in using a Rapid Subtraction Hybridization (RaSH) technique to identify genes differentially expressed during the interaction between tomato cells and either a protective *F. oxysporum* f. sp. *melonis* (Fom24) or its non protective mutant (rev 157). This technique enabled to identify sequences from both fungal and plant origin. Since data concerning fungal genes are scarce in Genbank, the most informative data concerned plant genes. Regarding fungi, the expression of two genes, a chitinase and a polyphenol oxydase, respectively involved in the degradation of cell walls and the oxidative catalysis of phenols was analyzed by Northern blot. Regarding plant genes, no newly expressed sequence was detected, demonstrating that the protective interaction results from a differential regulation of genes already expressed during the non protective interaction between plant cells and conidia of *F. oxysporum*. The expression profile of five genes was analyzed by Northern blot and real time PCR. Two of them are involved in the plant response to pathogen infection (*RIN4,* chitinase), and the three others are involved in important functions related to metabolism and transport (ATPase, ferredoxin-NADP-reductase and porin). The most

interesting result concerns the demonstration that *RIN4*, a gene implicated in a guard system, is involved in the interaction between cells of tomato of a susceptible variety and different strains of *F. oxysporum*.

USE OF MARKED STRAINS OF PATHOGENIC (FOL8) AND PROTECTIVE (FO47) *FUSARIUM OXYSPORUM* TO VISUALIZE THEIR INTERACTIONS WITH THE TOMATO ROOT IN SOIL. C. Olivain, C. Humbert, C. Steinberg and C. Alabouvette. *UMR Microbiologie du Sol et de l'Environnement, INRA, Université de Bourgogne, 21065 Dijon Cedex, France. E-mail: ala@dijon.inra.fr*

Several modes of action have been proposed to explain the biocontrol capacity of the *Fusarium oxysporum* protective strain Fo47, especially competition with the pathogen for root colonization. Confocal microscopy has been used to visualize the interactions between the transformed strains and the tomato root in soil. A disinfested soil was infested by Fo47 GFP, Fol8 DsRed2, or both fungi together at several concentrations before transplanting tomato seedlings. The general pattern of soil and root colonization was similar for the two fungi, but the saprophytic development of Fo47 was faster than that of the pathogenic strain. Conidia, at the surface of soil particles, germinated as early as 18 hours after transplantation of tomato. Hyphae reached the root surface where they created dense networks on the older part of the root, not showing any preferential growth. There was an obvious colonization of soil particles in the rhizosphere. In contrast to what was described in hydroponics, the elongation zone and the apices of tap and secondary roots were never colonized. When the two fungi were introduced together at the same concentration, both fungi were observed together in the older part of the root, but Fo47 was alone in the younger part of the root. When Fo47 was introduced at a higher inoculum concentration ($1x10^5$) than Fol8 ($1x10^3$), it obviously was dominant. However, it was possible to observe a few zones where both Fol8 and Fo47 were present together at the same spot. Fo47 never totally excluded Fol8 which growth was greatly restricted.

ANTAGONISTIC ACTIVITY OF SOME TELLURIC MICROORGANISMS ISOLATED FROM SUPPRESSIVE SOIL AND POSSIBILITY OF THEIR USE TO CONTROL BAYOUD DISEASE OF DATE PALM AND OTHER WILT DISEASES. My.H. Sedra. *Arab Organization for Agricultural Development (AOAD)/Institut National de Recherche Agronomique (INRA), Laboratory of Phytopathology,Genetics and Integrated control, Regional Centre, BP.533 Marrakech, Morocco. E-mail: sedramh@hotmail.com and mhsedra@yahoo.fr*

The bayoud disease on date palm caused by *Fusarium oxysporum* f. sp. *albedinis*, constitutes the most dangerous and threatening disease of date palm groves in the Arabian world and North Africa particularly. Several varieties of very high fruit quality were extinct and others can disappear because of their susceptibility to the disease. In order to preserve and to exploit the precious and/or rare date palm genotypes, the use of the suppressive soils and the application of the microbiological control seem to be possible. The antagonistic microorganisms isolated from suppressive soil were shown able to inhibit the spore germination, growth in soil and pathogenic activity of the pathogen on date palm plants, in comparison with non antagonistic microorganisms and some fungicides. The antagonists secrete substances that decrease the pathogen development significantly in water and in soil in comparison with fungicides and substances extracted from non antagonistic microorganisms. Some formulation substrates have been selected for their ability to store these microorganisms. In order to appreciate the effect of the antagonists on other diseases, the results showed that some antagonists were able to inhibit the growth of pathogens causing *Fusarium* wilt diseases of tomato, melon, asparagus, oil palm, pea and flax. In greenhouse, the biological trials showed that some antagonists decrease the wilt disease incidence of tomato and flax. It seems that the exploitation of these antagonistic microorganisms and the anti-fungal substances that they secrete becomes possible in order to control the studied diseases, particularly the Bayoud disease.

USE OF COMPOSTS FROM AGRICULTURAL AND URBAN WASTES AND *TRICHODERMA ASPERELLUM* (T34) TO SUPPRESS FUSARIUM WILT OF TOMATO. Mª I. Trillas[1], C. Borrero[2], S. Castillo[2], E. Casanova[1], G. Segarra[1] and M. Avilés[2]. *[1]Departamento de Biologia Vegetal, Universidad de Barcelona, Avenida. Diagonal 645, 08028 Barcelona, Spain. [2]Departamento de Ciencias Agroforestales, Universidad de Sevilla, Carretera de Utrera, Km. 1, 41013 Sevilla, Spain. E-mail: mtrillas@ub.edu*

Composts prepared from several wastes and used as plant growth media may have highly suppressive effects against diseases caused by a variety of plant pathogens. The most suppressive composts to tomato Fusarium wilt that we have evaluated are: grape marc composts, municipal organic and yard wastes compost and cork compost. Studies with grape marc and cork composts revealed a multiple correlation between disease severity and plant growth media pH and β-glucosidase activity. Therefore, pH and β-glucosidase activity were taken into account as predictive variable, more than 91% of the variation in severity of Fusarium wilt was explained. It was also reported that the microbial community from these suppressive plant growth media use more carboxylic acids, amino acids, amines, phenolic compounds and polymers than the microbial community from peat (conducive plant growth media). For both composts, the reduction of disease causes by *Fusarium oxysporum* f. sp. *lycopersici* in tomato plants was due to general and specific suppressiveness mechanisms. On the other hand, the biological control agent *Trichoderma asperellum* strain T34, was isolated from the compost obtained from organic and yard wastes. This isolate reduced significantly disease incidence when added to conducive peat, perlite and compost. Populations of *F. oxysporum* diminished in the presence of *T. asperellum* (T34). Nevertheless this isolate has higher populations in the presence of *F. oxysporum*.

EXPLORING THE INTERACTION BETWEEN BACTERIAL BIOCONTROL AGENTS AND GENETICALLY CHARACTERIZED MUTANTS OF *FUSARIUM OXYSPORUM*. D. Vitullo[1], F. De Curtis[1], A. Di Pietro[2] and G. Lima[1]. *[1]Dipartimento di Scienze, Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via F. de Sanctis, 86100 Campobasso, Italy. [2]Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain. E-mail: lima@unimol.it*

Biological control of soilborne plant pathogens represents a promising alternative to synthetic fungicides, with a number of bioformulates already available for practical use. However, the efficacy of biocontrol agents needs to be optimized to improve the reliability of biocontrol products and make them truly competitive with chemicals. The aim of this work is to gain a deeper understanding on the biochemical and molecular mechanisms underlying bacterial antagonism of soilborne fungi. Selected isolates of the genera

Bacillus and *Pseudomonas* obtained from suppressive soil were tested against a collection of genetically characterized mutants of the tomato vascular wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). Bacterial culture filtrates were fractionated according to molecular weight and tested for *in vitro* activity on mycelial growth and germ-tube elongation of the *Fol* wild type strain and different gene knockout mutants. Scanning electron microscopy was performed both *in vitro* and on tomato roots, to study the *in vivo* interaction among the bacterial cells of the most effective antagonists and the hyphae of the *Fol* wild type strain and selected fungal mutants. The results of this study will be discussed in relation to the possible modes of action involved in the tritrophic interaction between biocontrol bacteria, pathogen and host plant.

NOVEL BIOTROPHIC MYCOPARASITES FOR BIOLOGICAL CONTROL OF *FUSARIUM* PATHOGENS. V. Vujanovic. *Department of Food and Bioproduct Sciences, University of Saskatchewan, College of Agriculture and Bioresources, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8, Canada. E-mail: vladimir.vujanovic@usask.ca*

Fusarium pathogens cause multibillion dollar damages in North America, and greatly impact Canadian agriculture and forest economies. Saskatchewan's prairies represent 40% of Canadian cultivated land and are one of most important cereal crop regions worldwide. Seventeen indigenous fungal strains associated with Fusarium pathogens were isolated from Saskatchewan fields, preserved in Saskatchewan Microbial Collection and Database (SMCD), and used to study phylogeny of mycoparasites. DNA was extracted from these strains, and three different DNA fragments – including internal transcribed spacer (ITS), nuclear-encoded small subunit (nrSSU), and nuclear-encoded large subunit (nrLSU) – were amplified with ITS1/ITS4, NS1/NS6 and LS1/LR5 primer sets. Three different PCR fragments, 550 bp, 1150 bp, and 950 bp were observed with ITS1/ITS4, NS1/NS6, and LS1/LR5 primers, respectively. The amplified products were purified and sequenced. All sequences were aligned using ClustalW, and phylogenetic relationship was analyzed using neighbor-joining cluster analysis (PAUP). The cluster analyses revealed the biodiversity of six new biotrophic mycoparasites belonging to Sordariales and Pleosporales. These six strains were confronted with *F. avenaceum, F. graminearum, F. culmorum, F. proliferatum,* and *F. oxysporum* in co-culture trials. They were then considered for mycoparasitism, fungus-fungus, fungus-host-plant interactions and confocal microscopic studies.